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^HIV-3 RETROVIRUS ~~AND ITS USE~~ PROCESS FOR DETECTING

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source of virus.

Furthermore, the invention relates to a purified retrovirus having the essential morphological and immunological properties described below. In many cases, the unique characteristics of HIV-3 can best be appreciated by comparison with the same type of characteristics relating to the other human immunideficiency viruses, HIV-1 and HIV-2.

Brief description of the drawings

In Figures 1 to 16 the designations HIV-3 (ANT 70) and HIV-3 (ANT 70 NA) refer to two strains of a new HIV-3 virus isolated from a Camerounian woman and her partner from which HIV-3 (ANT 70) has been deposited under ECACC V88060301.

Figure 1 shows a procedure for preparing cleavage maps of viral proteins.

Figure 2 shows differential antigen capturing on virus-containing culture supernatants.

Differential antigen capturing is performed as described hereinafter. The solid line represents the results obtained using a broad-spectrum anti-HIV-1 IgG while the broken line depicts the results obtained using an IgG which was rather specific for HIV-1. ~~The titrations shown in panels A-E are typical for HIV-1. Panel F~~ shows the result obtained with HIV-3 (ANT 70) containing supernatant.

Figure 3A shows differential antigen capturing on HIV-1 and HIV-3 (ANT 70 NA) supernatants.

Figure 3B shows differential antigen capturing on Differential antigen capturing was performed as described hereinafter. The solid line depicts the results obtained on

Figure 2A, Figure 2B, Figure 2C, Figure 2D, and Figure 2E each show a typical titration obtained with HIV-1. Figure 2F

plates coated with the broad spectrum anti-HIV IgG while the broken line represents the results obtained on plates coated with IgG which shows less crossreactivity with HIV types other then HIV-1.

Figure 4A shows the reactivity of anti-HIV sera on HIV-1 and HIV-2 Western Blot strips.

↑ Figure 4B shows the reactivity of anti-HIV sera on

The reactivities of 3 different sera on HIV-1 and HIV-2 Western blot strips are shown. Sera: 1. anti-HIV-1, 2. anti-HIV-3 (ANT 70), 3. anti-HIV-2 (isolate 53). The molecular weights indicated are those given by the manufacturer (Dupont Biotech).

Figure 5 relates to the comparison of gag and pol proteins of several HIV-1 isolates, HIV-2rod and HIV-3 (ANT 70).

Proteins were separated electrophoretically and blotted as described later. The blot was incubated with a broad-spectrum anti- HIV antiserum followed by (anti-human IgG)/alkaline phosphatase- labeled conjugate to visualize the proteins.
A. HIV-2rod, B. an HIV-1 laboratory isolate, C. HIV-3 (ANT 70), D. an HIV-1 laboratory isolate, E. HIV-1 (SF4).

Figure 6 shows a comparison of HIV-3 (ANT 70) and HIV-3 (ANT 70 NA) proteins.

Proteins were separated electrophoretically and blotted as described later The blot was incubated with the BSR antiserum followed by (alkaline phosphatase)/anti-human IgG conjugate to visualize the proteins. Lane 1: HIV-3 (ANT 70 NA), lane 2: HIV-3 (ANT 70), lane 3: HIV-1 (SF4). The apparent intensity difference between lanes 1 and 2 is caused by the difference in the amount of material loaded.

Figure 7 relates to the ability of various human anti-HIV-1 sera to capture viral antigens.

A number of human sera were diluted 1:1000 and coated directly on microwell plates. Detergent-treated culture supernatants containing HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod or HIV-2 (isolate 53) were incubated and the bound antigen was detected using a broadspectrum (anti-HIV)/ horseradish peroxidase conjugate. Sera 1-7 were of African origin while sera 8-11 were from Europeans. The greater ability of African sera to capture non-HIV-1 antigen can, in part, be explained by their higher anti-p24 titers (data not shown).

Figure 8A, Figure 8B, Figure 8C and Figure 8D show ~~Figure 8 shows~~ the effect of coating IgG dilution on the binding of HIV isolates.
Succesive 2-fold dilutions were made of four different sera, beginning at a dilution of 1:1000 and were used to coat microwell plates. Detergent-treated supernatants of HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod and HIV-2 (isolate 53) were diluted to give approximately the same optical density on plates coated with the antiserum shown in panel B at a dilution of 1:1000. Bound antigen was detected using the broad-spectrum (anti-HIV IgG)/horseradish peroxidase conjugate.

Figure 9 shows antigen capturing of virus isolates using human polyclonal and mouse anti-HIV-1 monoclonal antibodies.

Wells were coated and incubated as described in the text. The IgGs used are as follows:

1. human polyclonal anti-HIV IgG , 2. MAb CLB 59, 3. MAb CLB

21, 4. MAb CLB 64, 5. MAb CLB 14, 6. MAb CLB 16, 7. MAb CLB 47, 8. MAb CLB 13.6 (anti-p18), 9. MAb CLB 19.7, 10. Mab CLB 13.4 (anti-p18).

Figure 10 ~~is a comparison~~ A-E show comparisons of the reactivity of human anti-HIV antisera to different HIV types.

Lysates of HIV-1 (SF4), HIV-3 (ANT 70), HIV-2rod and HIV-2 (isolate 53) were separated electrophoretically on SDS-polyacrylamide gels, blotted onto nitrocellulose, and incubated with a high titer anti-HIV-1 antiserum (panel A), a lower titer anti-HIV-1 antiserum (panel B), serum from the woman from whom HIV-3 (ANT 70) was isolated (panel C), her partner from which HIV-3 (ANT 70 NA) was isolated (panel D) and anti-HIV-2 antiserum from the person from whom HIV-2 (isolate 53) was isolated (panel E).

Figure 11 ~~shows~~ A-C show titrations of anti-HIV sera by enzyme immunoassay.

Microwell plates were coated with lysates of HIV-1 (SF4), HIV-3 (ANT 70) and HIV-2 (isolate 53). Serum from an HIV-1-infected European (~~left panel~~ Fig. 11A), antiserum to HIV-3 (ANT 70 NA) (~~center panel~~ Fig. 11B) and antiserum to HIV-2 (isolate 53) (~~right panel~~ Fig. 11C) were titrated in 2-fold dilutions beginning at a dilution of 1:100 on all three coated plates.

Figure 12 ~~shows~~ A and B show the positions of methionine and tryptophan residues in viral gag and pol gene products. p17 and p24 gene products and Figure 12C shows the positions of methionine and tryptophan residues in viral

Amino acid positions for the p17 gag proteins are given starting from the first methionine in the coding sequence. Positions for the p24 gag protein are given starting at the p17/p24 proteolytic cleavage site. Positions for the pol gene are shown after alignment with the highly conserved

tryptophan doublet in the HIV-1 sequence at positions 556 and 557. The positions of a conserved protease sequence, the protease/reverse transcriptase cleavage site and the reverse transcriptase/endonuclease cleavage site are indicated. In this case, the terms p24 and p17 are used in the genetic sense to refer to the largest and second largest viral core proteins respectively. The term "HIV-2 (LAV-2)" is a synonymum for HIV-2 rod.

Figures 13A-D show comparisons. ~~Figure 13 is a comparison~~ of partial cleavage products of gag and pol gene products of HIV-1 (SF4) [HIV-1 in the figure], HIV-3 (ANT 70) [isolate 70 in the figure], HIV-2rod [HIV-2 (LAV-2) in the figure] and HIV-2 (isolate 53) [isolate 53 in the figure]. The terms p24 and p17 are used in the genetic sense to indicate the largest and second largest viral core proteins, respectively.

Figures 14A-I, Figure 14A-II, Figure 14A-III, Figure 14B-I, Figure 14B-II, and Figure 14B-III show ~~Figure 14 shows~~ hybridization of cDNA probes to viral RNA.

Viral RNA from HIV-1 (SF4), HIV-2rod, and HIV-3 (ANT 70) were spotted onto a membrane filter as described in Materials and Methods. The filters were hybridized under either nonstringent (A) or stringent conditions (B) and autoradiographed.

1. Morphology

Electron microscopy of HIV-3-infected MT4 cells revealed the presence of extracellular virus particles having a diameter

antigenic differences between ANT 70 and HIV-1 are smaller than those between HIV-2 and HIV-1. This is particularly evident from the results presented in Figures 8 and 10.

Additional compelling evidence that ANT 70 is a unique virus different from HIV-1 and HIV-2 comes from the partial peptide maps. We have shown that there are significant differences in the most highly conserved viral proteins. The two HIV-2 isolates which were used for comparison gave essentially identical cleavage patterns except in the case of CNBr cleavage of the p17 core protein. It should be noted, however, that the p17 core protein exhibits more variability than the p24 protein, at least in HIV-1 strains (34). Whether or not this also holds true for HIV-2 awaits sequence determination on more strains than have been analyzed to date.

In light of the fact that ANT 70 is antigenically more closely related to HIV-1 than is HIV-2, as evidenced by a higher degree of crossreactivity which extends even to the gp41 envelope protein, was essential to establish that ANT 70 was more than simply a genetic variant of HIV-1. This was possible by investigating the locations of some of the most highly conserved amino acids in a number of viral proteins which are least subject to genetic variation. That major differences were noted in the cleavage patterns indicates that HIV-1, HIV-2 and ANT 70 are three genetically distinct viruses. On the other hand, the same series of experiments also revealed similarities between the viruses which may indicate that all three arose from a common progenitor.

The hybridization data also support the notion that ANT 70 is fundamentally different from either HIV-1 and HIV-2. As long as the conditions under which the hybridization is performed are stringent, a distinction can easily be made among ~~between~~ the three virus types. RNA of the HIV-3 retrovirus virtually hybridizes neither with the Env gene or the LTR close to it, in particular not with the nucleotide sequence 8352 - 9538 of HIV-1, nor with the sequences of the Pol region of the HIV-1 genome under stringent conditions.